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COMPOSITION OF THE BLOOD SERUM OF DEEP-SEA FISHES

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Abstract

Serum osmolarity, chloride, urea, protein, and trimethylamine oxide were measured in 15 shallow water marine teleosts, 6 elasmobranchs, 9 deep benthic teleosts, and 24 midwater teleosts. Amino acids, carbohydrates, phosphate, and hematocrits were determined for some species from these four groups. Elasmobranchs had high osmolarity (1035 mOsm/l) because of high serum urea (363 m*M*/l), TMAO (66 m*M*/l), and chloride (295 m*M*/l). Shallow water teleosts had low osmolarity (444 mOsm/l), chloride (176 m*M*/l), urea (4 m*M*/l), and TMAO (14 m*M*/l). Deep benthic teleosts had higher osmolarities and chloride levels (576 mOsm/l, 242 m*M*/l) than shallow water teleosts, as did midwater teleosts (561 mOsm/l, 267 m*M*/l). Serum TMAO was high in benthic (51 m*M*/l), but not midwater (12 m*M*/l) teleosts, and urea was low in midwater (1.0 m*M*/l) and benthic (1.5 m*M*/l) groups. Stress and morbidity raise osmolarity and chloride in marine teleosts and may account for high values in midwater and benthic fishes, which were sampled after considerable trauma. The data suggest that deep-sea teleosts osmoregulate as do shallow water species, and do not support the notion that osmoregulatory specializations, such as ureosmotic regulation, evolve more rapidly in the deep sea. Very low serum proteins (0.8 g/100 ml) and hematocrits (<10%) in midwater teleosts possibly relate to buoyancy or low metabolism.

Introduction

Marine fishes regulate their blood sera in three basic ways. Hagfish blood is isosmotic and, for most ions, isoionic to sea water. Blood osmolarities of sharks, their kin, and the coelacanth are similar to that of their environment, but their ion concentrations are lower because they maintain high levels of organic solutes such as urea and trimethylamine oxide (ureosmotic regulation). In the third pattern, found in marine teleosts and lampreys, blood serum is both hyposmotic and hypoionic to sea water (hyposmotic regulation).

The hagfishes' osmoregulation may not have changed from that of the earliest vertebrate ancestor, but the other two mechanisms depart radically from the condition in marine invertebrates, probably because these groups passed through a freshwater or estuarine evolutionary stage (Pang *et al.*, 1977). A number of reasons could explain why teleosts never developed ureosmotic regulation when they reinvaded the sea, even though this type of regulation probably evolved independently in the chondrichthians, the coelacanth, and the crab-eating frog, *Rana cancrivora* (Griffith and Pang, 1979). Among these possible reasons are: (1) They did not develop tolerance to elevated tissue urea levels. (2) Early teleost ancestors that entered the sea did not have enzymes of ureogenesis or had them in too low titers. (3) The teleosts evolved more recently than the chondrichthians and coelacanths,

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Abbreviations: WHOI, Woods Hole Oceanographic Institution; TMAO, trimethyl amine oxide.

and so did not have time to develop urea retention. (4) Ureosmotic regulation is only advantageous in a constant marine environment, and most teleosts whose blood chemistry has been studied inhabit inshore or estuarine areas during some stage in their life histories. (5) Ureosmotic regulation is energetically feasible only in large fishes, such as chondrichthians and *Latimeria*, which protect their young against passive urea loss during development. (6) Once the earliest marine teleosts developed hyposmotic regulation, changing to another osmoregulatory strategy was impossible because of evolutionary channelization.

Some of these hypotheses are testable. Despite a contrary report (Brown and Cohen, 1960), a variety of teleosts have all enzymes of the ornithine-urea cycles (Huggins *et al.*, 1969) and some have them in higher titers than certain urea-retaining chondrichthians (Read, 1967, 1971b). Although urea is moderately toxic to teleosts (Rasmussen and Rasmussen, 1977; Griffith *et al.*, 1979), it probably is not toxic enough at levels found in elasmobranch tissues to seriously impede evolution of ureosmotic regulation. Although the four remaining hypotheses are not subject to direct experimental proof, hypotheses 3 (insufficient time) and 4 (estuarine existence of most teleosts) can be tested indirectly by studying the body fluid chemistry of deep-sea teleosts.

Deep-sea fishes include many of the most ancient teleost lineages, providing a long evolutionary history in the sea, and they live in constant salinities with no exposure to a dilute environment in which urea retention would be a disadvantage. Because ureosmotic regulation in elasmobranchs uses less energy than hyposmotic regulation in teleosts (Griffith and Pang, 1979), urea retention should be of great advantage to teleosts in the deep sea, an energy-deprived environment where conservation of energy would have selective advantage.

Ureosmotic regulation is not the only possible osmoregulatory specialization deep-sea teleosts might have evolved to conserve energy. They could retain bulk quantities of organic solutes other than urea (e.g., trimethylamine oxide) to elevate blood osmolarity without raising blood electrolytes to toxic levels. Alternatively, they could build up ion (NaCl) concentrations to a condition like that in hagfishes, which are isosmotic and isoionic to sea water. Blaxter *et al.* (1971) suggested that, in contrast, midwater fishes might have lower blood solutes than other teleosts to provide buoyancy without using energy.

Few studies have dealt with osmoregulation in deep-sea fishes. Blaxter *et al.* (1971), testing whether midwater fishes use dilute body fluids for buoyancy, investigated blood osmolarity of several such fishes. Data also exist on the blood and tissue electrolytes of certain deep benthic fishes (Whitt and Prosser, 1971; Prosser *et al.*, 1975; Fänge *et al.*, 1972). However, no study has examined whether deep-sea fishes have evolved osmoregulatory specializations, such as urea retention, for coping with the constant high salinity and low available energy characteristic of their environment. This report seeks such specializations in a broad array of midwater and benthic deep-sea fishes.

Materials and Methods

All specimens were collected September 1974 to August 1975. The near-shore and surface oceanic fishes listed in Table I were collected in the vicinity of Woods Hole, Massachusetts (*Brevoortia, Opsanus, Gadus, Morone, Pomatomus, Stenotomus, Cynoscion, Scomber, Makaira, Palinurichthyes,* and *Paralichthyes*), near Bermuda (*Sphryaena*), or in the open ocean between Bermuda and Woods Hole (*Carynx, Cheilopogon,* and *Coryphaena*). Most were caught on hook and line, but

TABLE I

Blood serum constituents of some inshore marine and surface oceanic teleost fishes. (N = number of specimens tested for osmolarity, chloride, urea, protein and osmotic deficit, with TMAO usually measured in fewer. Condition: A = all specimens alive, D = all dead. Values are mean ± SE when N ⩾ 3, and mean alone when N < 3)

Species	N	Condition	Osmolarity (mOsm/l)	Chloride (m*M*/l)	Urea (m*M*/l)	Protein (g/100 ml)	TMAO (m*M*/l)	Osmotic deficit (m*M*/l)
Brevoortia tyrannus	2	A	408	181	1.7	3.4	8.0	36.3
Opsanus tau	10	A	333 ± 3	151 ± 5	3.4 ± 0.4	2.4 ± 0.2	4.8	22.8
Gadus morhua	1	A	416	162	2.3	5.7	6.9	82.8
Cheilopogon exsiliens	2	A	578	234	1.9	2.9	—	108.1
Morone saxatilus	6	A	424 ± 3	139 ± 7	10.4 ± 2.8	6.9 ± 0.5	19.2 ± 5.1	116.4
Pomatomus saltatrix	14	A	436 ± 4	159 ± 3	4.8 ± 0.8	6.6 ± 0.4	31.8 ± 11.0	81.4
Carynx chrysos	1	A	486	241	1.3	3.4	1.3	1.4
Coryphaena hippurus	8	A	464 ± 22	183 ± 13	4.3 ± 1.3	4.8 ± 0.5	14.5 ± 7.4	79.2
Stenotomus chrysops	12	A	370 ± 3	177 ± 2	2.0 ± 0.3	3.2 ± 0.2	37.4	−23.4
Cynoscion regalis	1	A	425	150	3.8	5.4	30.6	90.6
Sphyraena borealis	3	A	394 ± 9	144 ± 9	2.4 ± 0.7	6.7 ± 0.1	7.5	96.1
Scomber scomber	1	A	487	171	7.4	5.7	9.1	128.5
Makaira albidans	2	D	587	168	3.7	8.3	12.8	234.5
Palinurichthyes perciformis	1	A	492	208	9.0	5.3	5.8	61.2
Paralichthyes dentatus	3	A	363 ± 11	172 ± 15	2.1 ± 0.3	4.5 ± 0.4	10.6	6.8

Gadus and *Makaira* were collected using set long lines, *Cheilopogon* was caught in neuston nets, and *Opsanus* specimens were obtained from the Marine Biological Laboratory's specimen company. The elasmobranchs listed in Table II were col-

TABLE II

Blood chemistry of some marine elasmobranchs. (N = number of specimens tested for all factors except TMAO (for which fewer were generally tested). Condition: A = all specimens alive, D = all dead. Values are mean ± SE when N ⩾ 3 and mean alone when N < 3).

Species	N	Condition	Osmolarity (mOsm/l)	Chloride (m*M*/l)	Urea (m*M*/l)	Protein (g/100 ml)	TMAO (m*M*/l)	Osmotic deficit (m*M*/l)
Raja erinacea	3	A	991 ± 23	268 ± 13	416 ± 19	3.9 ± 0.4	41.4	2.4
Mustelus canis	2	A	1004	272	406	3.1	90.0	−36.0
Squalus acanthias	2	A	1005	275	368	3.2	22.0	65.0
Carcharhinus obscurus	1	A	1092	278	386	2.3	76.0	74
Prionace glauca	9	A	1036 ± 24	256 ± 4	391 ± 9	1.7 ± 0.2	99.0 ± 6	34
Centroscymnus coelolepis	1	D	1079	423	208	0.5	66.0	−41

lected using hook and line in the vicinity of Woods Hole (*Mustelus, Squalus,* and *Raja*), by set line on the continental shelf off the coast of Massachusets (*Prionace* and *Carcharhinus*), or by bottom trawl on the continental slope off New England (*Centroscymnus*). The deep benthic fishes listed in Table III were collected with otter or beam trawls in the Nares Abyss south of Bermuda at depths of *ca.* 5000 m (*Bathypterois*, a brotulid, and a rattail), on the continental slope off Massachusetts at depths of *ca.* 500 m (*Lepophidium, Macrozoarces, Urophycis,* and *Nematonurus*), or in Hudson's Canyon (*Nezumia* and *Antimora*). All the specimens reported as midwater fishes (Table IV) were collected using Issac-Kidd midwater trawls towed obliquely from 1000 m to the surface for about 2 h, mostly in the Sargasso Sea (many in, or near, cold core Gulf Stream rings; see Jahn, 1976, for details).

Identification of many of the species was problematical, particularly among the midwater and deep benthic groups, because workable keys were not available at sea and specimens could not be preserved. Expert identification of many of the midwater fishes was provided at sea by Drs. Andrew Jahn and James Craddock of Woods Hole Oceanographic Institution (WHOI). Species not positively identified are listed separately at the end of Table IV as "unidentified A", etc. Among the benthic fishes, *Nezumia bairdi* and *Antimora rostrata* were identified positively by Dr. Richard Haedrich (WHOI). The continental slope species (*Nematonurus, Urophycis, Lepophidium,* and *Macrozoarces*) and the abyssal *Bathypterois* were only tentatively identified. The unidentified abyssal rattail and brotulid are the same specimens reported on by Harvey and Steinhauer (1976). Fishes not positively identified were included in this study because their omission would impair generalizations that could be made about the blood chemistry of deep-sea fishes.

Blood of relatively large inshore teleosts and elasmobranchs was collected by puncturing the caudal artery with a large-gauge needle and withdrawing the blood into a heparinized syringe. The hearts of smaller specimens or moribund midwater

TABLE III

*Blood serum chemistry of some marine teleosts from the Continental Slope, Hudson's Canyon and abyss. (N = number of specimens tested for osmolarity, chloride, and osmotic deficit, with fewer often tested for urea, protein, and TMAO. Condition: D = all specimens dead at sampling. Values are means ± SE when N ⩾ 3, mean alone when N < 3. * = identification uncertain.)*

Group/species	N	Condition	Osmolarity (mOsm/l)	Chloride (m*M*/l)	Urea (m*M*/l)	Protein (g/100 ml)	TMAO (m*M*/l)	Osmotic deficit (m*M*/l)
Abyssal fishes								
Bathypterois sp.*	2	D	775	319	0.9	—	—	136
Rattail*	1	D	617	274	1.2	2.6	6.3	62
Brotulid*	1	D	591	236	0.7	—	—	118
Hudson's Canyon								
Nezumia bairdi	3	D	486 ± 41	227 ± 13	1.5 ± 0.1	2.0	—	31
Antimora rostrata	1	D	485	171	1.2	2.0	97	45
Continental slope								
*Nematonurus armatus**	8	D	567 ± 22	263 ± 15	1.3 ± 0.2	2.9 ± 0.4	49 ± 10	−9
*Urophycis tenuis**	3	D	608 ± 40	292 ± 22	1.1 ± 0.2	2.5	—	23
*Lepophidium cervinum**	1	D	538	203	4.0	—	—	128
*Macrozoarces americanus**	1	D	520	202	—	—	—	116

TABLE IV

Blood serum chemistry of some midwater marine teleosts. (N = number of specimens tested for osmolarity, chloride, and osmotic deficit, with fewer often measured for urea, protein, and TMAO. Condition: A = all specimens alive, D = all dead, AD = some alive and some dead. Values are mean ± SE when N ≥ 3, and mean alone when N < 3).

Species	N	Condition	Osmolarity (mOsm/l)	Chloride (m*M*/l)	Urea (m*M*/l)	Protein (g/100 ml)	TMAO (m*M*/l)	Osmotic deficit (m*M*/l)
Derichthys serpentinus	2	D	753	331	2.0	1.6	—	89
Nemichthys scolopaceus	4	D	706 ± 39	352 ± 23	0.9 ± 0.3	0.5	0.4	1
Bathylagus bericoides	1	A	374	155	0.1	—	—	64
Gonastoma elongatum	8	A	433 ± 14	204 ± 8	1.1 ± 0.2	0.5 ± 0.2	4.4 ± 1.0	20
Gonastoma elongatum	20	D	638 ± 30	313 ± 17	1.5 ± 0.0	0.5 ± 0.0	10.1	0
Argyropelycus aculeatus	10	D	578 ± 33	272 ± 19	1.3 ± 0.1	1.6 ± 0.1	22.8	10
Malocosteus niger	1	D	607	304	0.7	0.3	—	−2
Chauliodus sloani	8	AD	621 ± 73	306 ± 35	0.7 ± 0.2	0.5 ± 0.1	21.2 ± 9.7	−13
Stomias boa	2	D	427	217	—	—	—	−7
Echiostoma barbatum	2	D	463	184	—	—	35.0	60
Photonectes margarita	1	D	363	197	1.0	—	—	−32
Diaphus rafinesque	6	AD	550 ± 14	247 ± 13	1.5 ± 0.2	1.6 ± 0.2	14.7	40
Lampadena speculigera	1	D	462	198	0.8	—	—	65
Hygophum heigenmani	1	D	527	195	1.5	—	—	136
Ceratias holbrooki	1	A	545	275	0.8	0.4	6.2	−12
Anoplogaster cornuta	1	A	408	189	0.8	1.9	0.0	29
Scopelogadus beani	9	A	489 ± 33	239 ± 19	0.6 ± 0.2	0.4 ± 0.1	4.6 ± 0.5	6
Gempylus serpens	2	D	708	334	1.9	1.5	22.2	16
Nesiarchus nasutus	2	D	652	337	2.3	—	—	−24
Unidentified A	1	D	656	321	0.5	0.7	6.0	8
Unidentified B	2	D	668	336	0.8	0.3	16.8	−22
Unidentified C	2	D	604	298	1.2	0.4	19.2	−3
Unidentified D	1	D	608	312	0.9	0.4	6.6	−24
Unidentified E	1	A	522	256	0.7	0.4	3.3	6
Unidentified F	1	D	657	311	0.4	0.4	—	35

or benthic fishes were punctured and the blood collected directly in non-heparinized capillary tubes. Blood was refrigerated and later centrifuged to separate plasma or serum from red cells. The serum or plasma was then frozen at −20°C for subsequent analyses. Serum and plasma are both referred to as serum, since the

only difference expected between the two in the factors studied was slightly lower protein in serum. Hematocrits were routinely taken for most specimens.

Blood serum was analyzed using minor modifications of the microtechniques described in detail by Pickford *et al.* (1969). Serum osmolarity was determined on a Mechrolab (now Hewlett-Packard) vapor-pressure osmometer equipped with Hamilton microliter syringes which made it possible to analyze volumes of *ca.* 15 μl. Serum chloride was measured using an Aminco-Cotlove chloride titrator with sample volumes of 5 μl. Serum urea was estimated on 10 μl samples using the Hycel diacetyl monoxime method, with absorbance read on a Zeiss M4 QIII-PM QII spectrophotometer. Serum protein levels were determined spectrophotometrically by the method of Lowry *et al.* (1951). Trimethylamine oxide concentrations were estimated titrimetrically, in Conway diffusion dishes, by a modification of the technique of Forster *et al.* (1958). The reducing agent was metallic zinc, and the liberated free amine was absorbed in a boric acid buffer containing Bromcresol green indicator, and was titrated to a colorimetric end point with dilute HCl. Serum amino acids, carbohydrates, and phosphate were measured where the serum volume was sufficient. Amino acids were measured colorimetrically by the method of Troll and Cannon (1953); total carbohydrates by Anthrone reaction (Pickford *et al.*, 1969); and phosphate by the microtechnique of Lowry *et al.* (1953).

The contribution of unmeasured solutes to blood osmolarity can be approximated by adding the molar concentrations of two times chloride (*i.e.* assuming an equal number of monovalent cations to balance the chloride, and complete dissociation) to the other measured osmotically-active solutes (such as urea and trimethylamine oxide), and subtracting this sum from the measured osmolarity. These values of osmotic deficit do not include proteins, because of their uncertain osmotic contribution; nor do they include amino acids, carbohydrates, and phosphate, because of the limited number of species analyzed.

Because of the trauma to deep-sea fishes from net collection, most midwater and deep benthic specimens were in poor physiological condition and many were dead when sampled (Tables I–IV). Heartbeat was used to determine whether specimens were alive (A) or dead (D).

Results

The sampling problem

Comparing results from living and dead specimens of *Gonastoma elongatum* shows which serum constituents change after death and how much (Table IV). Dead *Gonastoma* specimens had significantly higher osmolarity (47%) and chloride (53%). Serum urea and protein did not change significantly. This relationship seemed to apply to most midwater teleosts; dead specimens generally had higher osmolarity and chloride, but urea and protein did not differ consistently. Dead midwater fishes generally had more trimethylamine oxide (TMAO) than those alive when sampled. The possible effects of pressure changes on a membrane's permeability to solutes and solvents has been explored by Gordon (1970) and MacDonald (1975), among others. No consistent effects have been demonstrated for midwater fishes (see also Whitt and Prosser, 1971; Prosser *et al.*, 1975).

Previous studies on stress in marine teleosts have shown marked increases in osmolarity and chloride, but have failed to show significant changes in protein levels (Umminger, 1970a; Fletcher, 1974; Forster and Berglund, 1956). Stress increases

renal urea excretion in elasmobranchs (Forster *et al.*, 1972), but no studies have demonstrated that stress changes blood urea levels in elasmobranchs or teleosts (review by Love, 1970). Among the constituents studied in only a few species, total carbohydrates are almost certainly elevated by stress (Chavin, 1964; Wedemeyer, 1972); phosphate may be affected by stress but the direction of the change is equivocal (Forster and Berglund, 1956; Hammond and Hickman, 1966); and little is known of how amino acids respond to acute stress in fishes.

Variation within groups

Shallow water teleosts. Blood sera of shallow water teleosts (Table I) are hyposmotic, with osmolarities (mean = 444; range = 333–587 mOsm/l) roughly 40% of sea water. All except the high values in *Makaira* and *Cheilopogon* and the low ones of *Opsanus* were close to the range (386–476 mOsm/l) compiled by Holmes and Donaldson (1969) for marine teleosts. Fletcher (1974), among others, has shown that post-mortem changes and stress can raise osmolarity in teleosts, which could account for the high values above. Serum chloride values (mean = 176; range = 139–234 m*M*/l) varied only slightly and were within the range of values compiled for marine teleosts by Holmes and Donaldson (1969) and Prosser (1973). The blood protein levels (mean = 5.0; range = 2.4–8.3 g/100 ml) were mostly in the range given by Holmes and Donaldson (1969) for comparable shallow water teleosts (2.8–8.0 g/100 ml). Serum urea levels (mean = 4.0; range = 1.3–10.4 m*M*/l) are low, as previously reported (Holmes and Donaldson, 1969).

Although TMAO in teleost serum has generally been regarded as negligible or undetectable (Norris and Benoit, 1945; Lange and Fugelli, 1965; Nicol, 1967), the present findings suggest it is a significant solute in the blood sera of several shallow water teleosts, including *Pomatomus, Stenotomus,* and *Cynoscion* (mean = 14; range = 1–37 m*M*/l). Diet, stress, season, or other factors may have contributed to these high values (Love, 1970).

The osmotic deficit was quite high for some species (*e.g.* over 100 m*M*/l in *Cheilopogon, Morone, Scomber,* and *Makaira*), suggesting they may contain substantial quantities of unidentified solutes. Teleost fishes, like tetrapods, often have large cation excesses (Pickford *et al.*, 1969) in which chloride levels are much lower than the levels of cations such as sodium and potassium because of large amounts of bicarbonate, phosphate, amino acids, lactate, and protein. The low osmotic deficit of *Stenotomus* (excess of 23 m*M*/l) may be an artifact of the very high TMAO concentration in the one specimen measured. In at least some shallow water teleosts, amino acids (mean = 12.4; range = 5–25 m*M*/l) may contribute substantially to osmolarity (Table V). Total carbohydrates (mean = 175; range = 54–385 mg/100 ml) probably are minor osmotic constituents in unstressed fish, and phosphate levels (mean = 5.6; range = 4.6–6.5 m*M*/l) also are relatively minor osmotically. The levels of amino acids, carbohydrates, and phosphate, except the very high carbohydrate concentration in *Pomatomus*, agree with published values in teleosts (summarized by Pickford *et al.*, 1969, and Griffith *et al.*, 1974).

Marine elasmobranchs. All of the elasmobranchs studied except *Centroscymnus* (Table II) had consistent osmolarities (991–1092 mOsm/l; mean = 1035) similar to that of sea water, chloride levels of 256–278 m*M*/l, high urea (368–416 m*M*/l), and protein levels of 1.7–3.9 g/100 ml. These data agree with most published reports on the blood chemistry of marine elasmobranchs (reviews by Smith, 1936; Holmes and Donaldson, 1969; Pang *et al.*, 1977). The high osmolarity and chloride and low protein and urea in *Centroscymnus* (Table II) may be an artifact of post-

TABLE V

Other blood serum constituents in some marine fishes. (Values are mean ± SE when N ≥ 3 and mean alone when N < 3. Sample size is in parentheses.)

Group/species	Amino acids (m*M*/l)	Carbohydrates (mg/100 ml)	Phosphate (m*M*/l)
Shallow Water Teleosts			
Brevoortia tyrannus	8.9 (2)	121 (2)	4.6 (1)
Gadus morhua	5.0 (1)	54 (1)	6.4 (1)
Pomatomus saltatrix	11.1 ± 1 (3)	385 ± 81 (5)	6.5 (1)
Sphyraena borealis	24.5 (2)	138 (2)	5.0 (1)
Elasmobranchs			
Raja erinacea	3.5 (1)	129 (1)	—
Squalus acanthias	4.2 (1)	—	—
Prionace glauca	—	133 (2)	—
Centroscymnus coelolepis	18.9 (1)	42 (1)	8.0 (1)
Benthic Teleosts			
Abyssal rattail	34.2 (1)	121 (1)	6.2 (1)
Nematonurus armatus	12.1 (2)	44 (2)	6.2 (2)
Urophycis tenuis	20.6 (2)	44 (2)	3.3 (2)
Midwater Teleosts			
Malocosteus niger	—	62 (1)	—
Chauliodus sloani	6.7 (1)	47 (1)	5.8 (1)
Anoplogaster cornuta	13.2 (1)	124 (1)	8.2 (1)
Scopelogadus beani	4.5 (2)	9 (2)	1.8 (1)

mortem changes or it may be due to a deep-sea habitat, since the deep water holocephalans have similar blood serum chemistry (Fänge and Fugelli, 1963; Robertson, 1976; Read, 1971a).

Reported serum TMAO levels of elasmobranchs are high (reviews by Pang *et al.*, 1977; Holmes and Donaldson, 1969; Love, 1970; Nicol, 1967; Forster and Goldstein, 1969). The levels in this study were substantial but varied (range = 22–99; mean = 66 m*M*/l). The elasmobranch osmotic deficits generally were low, from an excess of 41 m*M*/l in *Centroscymnus* to a deficit of 65 m*M*/l in *Squalus*, suggesting most elasmobranchs do not contain unidentified solutes in large amounts. Serum amino acids (mean = 8.9; range = 3.5–18.9 m*M*/l) and carbohydrates (mean = 101; range = 42–183 mg/100 ml) were relatively insignificant osmotically and agreed with published data (Keirmeir, 1939; Denis, 1922; Brull, 1956; Smith, 1929). *Centroscymnus* phosphate (8 m*M*/l; Table V) was higher than previously reported for other elasmobranchs (Holmes and Donaldson, 1969).

Deep benthic teleosts. Serum osmolarities in deep benthic teleosts studied here (Table III) range from unexceptional levels of around 486 in *Nezumia* and *Antimora* to very high (albeit hyposmotic to sea water) levels of 775 mOsm/l in *Bathypterois*. Similarly, chloride ranged from moderate values of *ca.* 200 m*M*/l in some species to 319 m*M*/l in *Bathypterois*. Urea levels (0.9–4.0; mean = 1.5 m*M*/l), and protein levels (2.0–2.9; mean = 2.4 g/100 ml) varied little. TMAO concentrations ranged from a low of 6.3 m*M*/l in the abyssal rattail to a high in *Antimora* of 97 m*M*/l. Published data on electrolytes in rattails (Fänge *et al.*, 1972; Whitt and Prosser, 1971; Prosser *et al.*, 1975) show values generally similar to those found here. Many benthic species examined here had large osmotic deficits (4 species over 100 m*M*/l), suggesting that their blood contained considerable unidentified solutes. The high amino acids in some species (mean = 22; range = 12–34 m*M*/l) may have contributed to the unexplained osmolarity. Carbohy-

drates (mean = 70; range = 44–101 mg/100 ml) and phosphate (mean = 5.2; range = 3.3–6.2 m*M*/l) were probably of less consequence (Table V).

Midwater teleosts. Osmolarity and chloride of midwater teleost fishes (Table IV) ranged widely (363–753; mean = 561 mOsm/l osmolarity), (184–352; mean = 267 m*M*/l chloride), although all species were hyposmotic and hypoionic to sea water. Blood urea, ranging from 0.1–1.9 m*M*/l (mean = 1.0 m*M*/l) was a somewhat variable but osmotically insignificant solute in all species tested, and blood proteins (0.3–1.9 g/100 ml; mean = 0.8 g/100 ml) tended to be low, though variable. Serum TMAO concentrations (0.0–35 m*M*/l; mean = 12 m*M*/l) were variable and were a consequential osmotic solute in some species such as *Echiostoma, Chauliodus, Gempylus,* and *Argyropelycus.* The osmotic deficit (−32 to 136 m*M*/l) was low in most species, indicating that the measured solutes gave a good picture of overall serum osmolarity. Serum amino acids (mean = 8.1; range = 6.7–13.2 m*M*/l), carbohydrates (mean = 61; range = 9–124 mg/100 ml), and phosphate (mean = 5.3; range = 1.8–8.2 m*M*/l) although measured in a few species (Table V), did not appear to be important osmotic solutes. Blaxter *et al.* (1971) indicate that midwater teleosts have a wide range of osmolarities (*ca.* 365–1000 mOsm/l, estimated from freezing point depression), with the highest value isosmotic with sea water. Blaxter *et al.* (1971) also point out that many midwater fishes, especially those lacking swim bladders, had high water content. This is consistent with the low serum protein levels reported here.

Comparison of serum solutes in the various groups of marine fishes

Osmolarity. The osmolarity of the blood serum of elasmobranch fishes (1035 mOsm/l, Table VI) was much higher than that found for the teleosts. It was consistently isosmotic or somewhat hyperosmotic to the sea water environment (generally *ca.* 1000 mOsm/l; Prosser, 1973). Within the teleosts, the osmolarities of both the midwater and deep benthic fishes were similar (561 and 576 mOsm/l, respectively), and higher, though not significantly so at the 5% confidence level, than those of the shallow water species (444 mOsm/l). The differences between the hyposmotic teleosts and the isosmotic elasmobranchs have been reviewed many times in the literature (Pang *et al.*, 1977; Smith, 1936; Smith, 1953; Holmes and Donaldson, 1969; Conte, 1969; etc.). It is questionable whether the trends found here, for the midwater and deep benthic fishes to have higher osmolarities than the shallow water species, are meaningful. The deep-sea fishes were mostly sampled while dead, whereas the shallow water fishes were mostly sampled alive, and post-mortem changes and stress probably lead to elevated blood osmolarities in teleosts. However, some midwater, deep benthic, and shallow water teleosts may possess fairly high osmolarities in the natural state, though none approach being isosmotic with the environment.

Chloride. Blood chloride levels were highest in elasmobranchs (295 m*M*/l). These elasmobranch levels were significantly higher than those of shallow water teleosts (176 m*M*/l), but did not differ significantly from those of the deep benthic (243 m*M*/l) or midwater (267 m*M*/l) teleosts (Table VI). None of the teleost groups differed significantly from one another, although the deep-sea groups tended to have higher chloride levels than the shallow water group. Elasmobranchs generally have higher serum ion levels than (shallow water) teleosts (reviews by Holmes and Donaldson, 1969; Pang *et al.*, 1977). The generally higher chloride levels in

TABLE VI

*A comparison of the blood serum composition of different groups of marine fishes. (Values are the means of the means for species from Tables I–V ± SE, with the ranges of species' means in parentheses. * = Significantly different from shallow water teleosts, P < .05, using Student's* t *test.)*

	Osmolarity (mOsm/l)	Chloride (m*M*/l)	Urea (m*M*/l)	Protein (g/100 ml)	TMAO (m*M*/l)	Amino Acids (m*M*/l)	Carbohydrates (mg/100 ml)	PO_4 (m*M*/l)
Shallow water teleosts	444 ± 19 (333–587)	176 ± 8 (139–241)	4.0 ± 0.7 (1.3–10.4)	5.0 ± 0.4 (2.4–8.3)	14.3 ± 3.0 (1.3–37.4)	12.4 ± 4.2 (5.0–24.5)	175 ± 73 (54–385)	5.6 ± 0.5 (4.6–6.5)
Marine elasmobranchs	1035 ± 17* (991–1092)	295 ± 26* (256–423)	363 ± 32* (208–416)	2.5 ± 0.5 (0.5–3.9)	65.7 ± 12.0 (22–99)	8.9 ± 5.0 (4.2–18.9)	101 ± 30 (42–133)	8.0
Deep benthic teleosts	576 ± 30 (487–775)	243 ± 16 (171–319)	1.5 ± 0.4 (0.7–4.0)	2.4 ± 0.2 (2.0–2.9)	50.7 ± 26.2 (6.3–97)	22.3 ± 6.4 (12.1–34.2)	70 ± 26 (44–121)	5.2 ± 1.0 (3.3–6.2)
Midwater teleosts	561 ± 22 (363–753)	267 ± 12 (184–352)	1.0 ± 0.1 (0.1–2.3)	0.8 ± 0.1* (0.3–1.9)	12.1 ± 2.5 (0.0–35.0)	8.1 ± 2.6 (6.7–13.2)	61 ± 24 (9–124)	5.3 ± 1.9 (1.8–8.2)

deep-sea fishes may indicate that higher blood ions conserve energy, or may be an artifact of trawling stress and post-mortem changes.

Urea. Only elasmobranchs, among the fishes studied, had high urea levels (363 m*M*/l; Table VI). All marine elasmobranchs retain high levels of urea (Smith, 1936; Pang *et al.*, 1977; Holmes and Donaldson, 1969). This is also characteristic of holocephalans (Read, 1971a; Fänge and Fugelli, 1963; Robertson, 1976) and the coelacanth (Pickford and Grant, 1967; Lutz and Robertson, 1971; Griffith, 1980). In these groups urea brings osmolarity close to that of sea water while maintaining blood electrolytes at levels much lower than sea water. No teleost species studied in the present report had urea levels high enough to act as a substantial osmotic solute. Deep water teleosts may have lower urea levels than shallow water species, although the differences were not statistically significant.

Protein. Serum protein levels tended to be highest in shallow water teleosts, intermediate in elasmobranchs and deep benthic teleosts, and lowest in midwater teleosts (Table VI). Protein in the midwater teleosts was much lower than in inshore teleosts, but no other difference between groups in serum protein levels was significant. The low protein levels in midwater teleosts may be due to their need to maintain neutral buoyancy with minimal energy, resulting in reduction of hard skeletal material and heavy proteins. Such reduction in midwater fishes, especially those lacking a gas bladder, has been documented by Denton and Marshall (1958) and Blaxter *et al.* (1971). The lower serum protein levels in elasmobranchs and deep benthic teleosts than in shallow water teleosts are probably not related to buoyancy. These levels may relate to activity, since within the shallow water teleosts, active fish tend to have higher serum protein than inactive fish like *Opsanus.*

Trimethylamine oxide. Serum TMAO levels varied within all four groups of fishes studied, and comparisons among the groups showed no significant differences (Table VI). Most elasmobranchs, however, had high TMAO levels, consistent with the osmoregulatory role usually ascribed to TMAO in these fishes (reviews by Smith, 1936; Groninger, 1959; Pang *et al.*, 1977). A number of teleosts also had high TMAO levels that presumably contribute substantially to their total serum osmolarity. No clear relationships between habitat and TMAO concentrations were

evident. Differences in diet might account for some of the variability, since diet is teleosts' principal source of TMAO (Norris and Benoit, 1945).

Amino acids, carbohydrates, and phosphate. Differences in serum amino acids, carbohydrates, and inorganic phosphate levels were neither consistent nor significant in the four groups studied (Tables V and VI), although too few species for generalization may have been tested. In certain species, amino acids contributed substantially to osmolarity (*e.g.*, levels over 15 m*M*/l in *Sphyraena, Centroscymnus, Urophycis*, and the abyssal rattail), but the contributions of carbohydrates (a maximum of *ca.* 21 m*M*/l in the hyperglycemic *Pomatomus*, and probably less in unstressed animals) and inorganic phosphate (the highest was 8.2 m*M*/l) were probably not important to total serum osmolarity.

Hematocrits. Hematocrits in the few inshore teleosts we measured, mostly active species such as *Scomber, Coryphaena,* and *Carynx*, were around 50%. Hematocrits in elasmobranchs were lower (5–29%), in agreement with Wintrobe (1934). The deep benthic teleosts had values of 15–40% (average = 25%), and the lowest hematocrits were in the midwater teleosts (2–30%, but none over 10% except *Argyropelycus* and the myctophid species studied). Although stress and morbidity affect hematocrit, the change usually is an increase (Fletcher, 1974), suggesting that our low values are realistic. Blaxter *et al.* (1971) observed similar low hematocrits (5–9%) in midwater fishes lacking swim bladders, and suggested that this was due to low activity.

Discussion

Griffith *et al.* (1973) suggested that deep-sea teleosts evolved urea retention to conserve energy in osmoregulation. The present data do not support this hypothesis. No midwater or deep benthic teleost investigated had levels of urea above 4 m*M*/l; most had less than shallow water teleosts. Although Griffith *et al.* (1973) further suggested that urea retention would be even more advantageous to viviparous or ovoviviparous deep-sea fishes such as brotulids or zoarcids, the abyssal brotulid tested had low urea (0.7 m*M*/l) and the serum of shallow water *Macrozoarces americanus*, an oviparous relative of the viviparous zoarcids, also had negligible urea concentrations (Griffith, unpublished). In light of the large number of species from a variety of habitats found to lack the mechanism, it seems likely that no teleost possesses ureosmotic regulation.

One alternative to the urea retention hypothesis is that some deep-sea teleosts could build up ion (NaCl) concentrations to sea water levels and conserve energy in both osmotic and ionic regulation. Although high osmolarity, mostly accounted for by chloride (plus sodium), exists in a variety of deep-sea fishes, no teleost studied reached the isosmotic, isoionic condition found in hagfishes. The highest teleostean osmolarities and chloride levels found here were 775 mOsm/l and 319 m*M*/l in the benthic *Bathypterois*, and 753 mOsm/l and 331 m*M*/l in the midwater eel, *Derichthys*. These contrast with the hagfish, *Myxine*, with an osmolarity of 1034 mOsm/l and chloride levels of 508 m*M*/l (Robertson, 1966; Munz and McFarland, 1964). Some deep-sea teleosts may be evolving the hagfish mechanism, but the generally poor physiological condition of all deep-sea fishes studied and the fact that trawling stress and morbidity elevate chloride and osmolarity in marine teleosts (Fletcher, 1974; Umminger, 1970a; Forster and Berglund, 1956) make the results of the present study unclear. The lowest osmolarities and chloride levels in midwater fish specimens were from those in the best physiological condition, a finding also evident from Blaxter *et al.* (1971).

Deep-sea fishes could elevate blood osmolarity without raising blood electrolytes to toxic levels nor developing special mechanisms to retain urea, by building up organic solutes such as amino acids, carbohydrates, or other metabolic products. The best osmotic solutes (1) would be non-toxic, readily supplied through common metabolic pathways or the diet, (2) should not be crucial to energy or synthetic metabolism, and (3) should not pass easily through membranes between the fish and its environment. TMAO, retained in addition to urea in marine elasmobranchs, is one example of this sort. Although vertebrates' use of amino acids or carbohydrates as extracellular solutes in osmoregulation has not been documented, many invertebrates and some vertebrates do build up these compounds in response to low temperatures (Umminger, 1970b; Prosser, 1973).

The present data suggest some deep-sea teleosts might use TMAO and amino acids to elevate osmolarity. Among benthic deep-sea teleosts high TMAO (over 40 m*M*/l) is found in *Antimora* and *Nematonurus*, and high amino acids (over 20 m*M*/l) in *Urophycis* and an abyssal rattail. Four species (*Bathypterois, Lepidophidium, Macrozoarces*, and an abyssal brotulid) have osmotic deficits of more than 100 m*M*/l, suggesting abundant non-measured solutes that may include TMAO and amino acids. Among midwater fishes these compounds seem to be less important, most species having low osmotic deficits and only a few having osmotically significant levels of TMAO or amino acids. A variety of shallow water teleosts have substantial osmotic deficits, TMAO levels, or amino acids. No species studied, however, had levels of extra solutes high enough to bring the total osmolarity close to that of sea water. Stress and morbidity might break down the cellular barrier, releasing TMAO, amino acids, lactate, etc., and thus raising concentrations of these substances in the blood. Hence, this hypothesis is dubious and of limited importance.

Blaxter *et al.* (1971) suggested that midwater fishes, particularly those lacking swim bladders, might reduce total solute concentrations in their body fluids to help achieve neutral buoyancy. The present data confirm the results of Blaxter *et al.* (1971), but fail to substantiate their hypothesis. The midwater fishes studied here generally had higher total solutes, as reflected in total osmolarity, than did shallow water teleosts. The buoyancy hypothesis, however, may partially explain why midwater fishes have few red blood cells and low concentrations of particularly heavy solutes, such as proteins. The need for neutral buoyancy might have inhibited midwater fishes from developing osmoregulatory specializations that involve accumulating extra solutes.

Thus, deep-sea teleosts appear to osmoregulate by the same mechanisms used by their shallow water relatives. Although adaptation to the deep-sea environment resulted in the evolution of extraordinary physiological and/or morphological specializations in nearly every functional system in some fishes (general reviews by Gordon, 1970; Brauer, 1972; MacDonald, 1975; and Locket, 1977), no such interesting specializations have developed in teleostean osmoregulation. The reasons that novel mechanisms did not develop are probably complex and may involve such factors as buoyancy. However, once vertebrates acquired low blood electrolytes and once teleosts had adapted to marine environments by hyposmotic rather than ureosmotic regulation, changing to another osmoregulatory strategy may have become impossible.

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